

On the Weight of Precipitum Obtainable in Precipitin Interactions with Small Weights of Homologous Protein

D. A. Welsh and H. G. Chapman

Proc. R. Soc. Lond. B 1908 **80**, 161-164
doi: 10.1098/rspb.1908.0016

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

To subscribe to *Proc. R. Soc. Lond. B* go to:
<http://rspb.royalsocietypublishing.org/subscriptions>

On the Weight of Precipitum obtainable in Precipitin Interactions with Small Weights of Homologous Protein.

By Professor D. A. WELSH and Dr. H. G. CHAPMAN.

(Communicated by Dr. C. J. Martin, F.R.S. Received December 17, 1907,—Read February 6, 1908.)

(From the Physiological and Pathological Laboratories of the University of Sydney.)

In a previous communication* on precipitin reactions, we brought forward observations which led to the conclusion that the precipitum is derived mainly from the antiserum and not from the homologous protein. In a recent review† of our paper the suggestion was made that gravimetric evidence might be more conclusive. Acting on this suggestion, we have carried out experiments in which a considerable amount of antiserum was allowed to interact with a known small amount of homologous protein. As soon as the interaction was completed, the deposits were collected, washed, and weighed. In every case the amount of dried precipitum exceeded the amount of dried homologous protein. The smallest precipitum obtained weighed more than twice, the largest more than 25 times, the homologous protein employed in the interaction. Former observations‡ had revealed that antisera require very different amounts of protein to ensure the formation of maximal deposits. It was, therefore, to be expected that considerable variations would be obtained in the weight of deposits produced by small amounts of protein in different antisera.

Method of Experiment.

The antiserum was obtained from rabbits which were bled on the day of the experiment. One milligramme or 2·5 milligrammes dried egg-white or blood serum was dissolved in 10 c.c. salt solution and added to the homologous antiserum, together with about 90 c.c. salt solution. The whole of the available clear antiserum, with the exception of less than 1 c.c. reserved for control observations, was employed. The dried protein was prepared under aseptic conditions, and every precaution was taken to exclude micro-organisms§

* Welsh and Chapman, 'Roy. Soc. Proc.,' B, vol. 78, p. 297, 1906.

† Mouton, 'Bulletin de l'Institut Pasteur,' 1907.

‡ Welsh and Chapman, 'Journ. of Hygiene,' vol. 6, p. 263, 1906.

§ We are unable to confirm Friedberger, 'Centralb. f. Bakt., Orig.,' vol. 43, pp. 490—494, March 5, 1907, in his statements regarding the prevention of bacterial growth by precipitin antisera, as our tubes tend to become infected in about 7 days.

during the experiment. After 48 hours the clear superfluids were removed, and were usually subjected to further experiment, either by addition of more homologous protein, or by addition of fresh antiserum from another immunised animal. Since it had been shown* that different homologous antisera might interact to yield a precipitate, appropriate controls were carried out which showed that no trace of deposit occurred as the result of the interactions with one another of the antisera used in these experiments.

The deposits from the interactions were shaken up three times with 50 c.c. saline solution, and separated by the centrifuge. They were then washed five times with 50 c.c. distilled water, whereupon the superfluid yielded no trace of soluble protein. After three washings with absolute ethyl alcohol, they were finally treated with ether free from water. On removal of the excess of ether with a pipette, the deposits were placed in an oven at 50° C. for 24 hours, and dried to constant weight in a desiccator over calcium chloride. The weighings were made in small glass test-tubes of about 7 grammes tare, in which the final washings with alcohol and with ether had been carried out.

Amount of Precipitum obtainable from Large Quantities of Antiserum with Small Amounts of Homologous Protein.

Five experiments were undertaken, and the results are summarised in Table I.

Table I.

No. of experiment.	Weight of homologous protein (dried).	Amount of antiserum (fresh).	Weight of precipitum (dried).
			milli-grammes.
1	2.5 milligrammes hen egg-white.....	19 c.c. hen egg antiserum (No. 40) ...	18.0
2	2.5 milligrammes hen egg-white.....	20 c.c. hen egg antiserum (No. 41) ...	18.0
3 (a)	1.0 milligramme horse serum	19 c.c. horse antiserum (No. 43)	2.2
3 (b)	Superfluid of 3 (a)	13 c.c. horse antiserum (No. 42)	5.0
4 (a)	1.0 milligramme ostrich egg-white...	20 c.c. ostrich egg antiserum (No. 44) ...	5.0
4 (b)	4.0 milligrammes ostrich egg-white	Superfluid of 4 (a)	11.0
5 (a)	1.0 milligramme hen egg-white	18 c.c. hen egg antiserum (No. 47) ...	13.0
5 (b)	Superfluid of 5 (a)	16 c.c. hen egg antiserum (No. 46) ...	8.5
5 (c)	Superfluid of 5 (b)	18 c.c. hen egg antiserum (No. 48) ...	4.4

In experiment (1) 2.5 milligrammes dried hen egg-white interacted once with 19 c.c. fresh hen egg antiserum (No. 40) and caused the formation of a deposit

* Nuttall, Hopkins, and Strangeways, quoted by Nuttall, 'Blood Immunity and Blood Relationship,' p. 129, Cambridge, 1904.

1907.] *Precipitum obtainable in Precipitin Interactions, etc.* 163

weighing 18 milligrammes, or more than seven times the weight of the homologous protein concerned.

In experiment (2) also 2.5 milligrammes dried hen egg-white interacted once with 20 c.c. fresh hen egg antiserum (No. 41), and yielded a precipitum of 18 milligrammes.

In experiment (3) 1 milligramme dried horse blood serum, interacting (*a*) with 19 c.c. fresh horse antiserum (No. 43) produced a deposit of 2.2 milligrammes, or more than twice the weight of the homologous protein. The residual homologous protein in the clear superfluid, interacting (*b*) with 13 c.c. of a second fresh horse antiserum (No. 42), yielded a further deposit of 5 milligrammes, making 7.2 milligrammes in all, or more than seven times the amount of the original homologous protein.

In experiment (4) (*a*) 1 milligramme dried ostrich egg-white, interacting with 20 c.c. fresh ostrich egg antiserum (No. 44), led to a deposit of 5 milligrammes, and (*b*) the subsequent addition of 4 milligrammes dried ostrich egg-white to the clear superfluid led to a further deposit of 11 milligrammes.

In experiment (5) 1 milligramme dried hen egg-white, interacting (*a*) with 18 c.c. fresh hen egg antiserum (No. 47), brought down a deposit of 13 milligrammes. The residual protein in the clear superfluid, interacting (*b*) with 16 c.c. of a second fresh hen egg antiserum (No. 46), gave a further deposit of 8.5 milligrammes. The resulting superfluid, again interacting (*c*) with 18 c.c. of a third hen egg antiserum (No. 48), gave a third deposit of 4.4 milligrammes. Thus, by allowing 1 milligramme of the homologous protein to interact successively with three antisera (which did not mutually interact), a total deposit of 25.9 milligrammes was attained.

The progressively diminishing weights of precipitum obtained from the successive antisera in experiment (5) (*a*), (*b*), and (*c*), do not necessarily indicate that the homologous protein is being appreciably used up in the interactions. A difference in weight of deposit may be an indication merely of a difference in precipitability of antiserum, a circumstance to which we have already referred. This interpretation is consistent with the control observations made with each of these antisera and fresh homologous protein, and also consistent with the progressively increasing weights of deposit obtained in similar circumstances in experiment (3) (*a*) and (*b*) and its corresponding controls. Thus in the control observations in experiment (5), when 0.1 c.c. of each of the three antisera concerned was tested separately with 0.00005 gramme homologous protein, it was found that the largest deposit was given by (*a*) antiserum No. 47, and the smallest by (*c*) antiserum No. 48.

164 *Precipitum obtainable in Precipitin Interactions, etc.**Conclusions.*

These observations reinforce our previous conclusions* regarding certain phenomena of precipitin reactions by showing :—

(1) That the homologous protein is capable of throwing out of solution many times its weight of precipitum, and

(2) That, in the interaction of minute quantities of homologous protein with large amounts of antiserum, the protein is not completely removed from the superfluid, but that sufficient remains to produce a second, and also to produce a third interaction with similar large amounts of fresh antiserum.

It is apparent, therefore, that the serum (antiserum) of a rabbit, immunised with egg-white or with blood serum, acquires the capacity, not so much of precipitating, as of being precipitated by the homologous protein; in other words, that the antiserum, and not the homologous protein, is the main source of "precipitable" substance.

We desire to acknowledge the courtesy of Professor Anderson Stuart in placing his laboratory at our disposal.

* Welsh and Chapman, 'Roy. Soc. Proc.,' B, vol. 78, p. 312, 1906.
